2

Lxra deficiency hampers the hepatic adaptive response to fasting in mice

M.H. Oosterveer
T.H. van Dijk
A. Grefhorst
V.W. Bloks
H. Havinga
F. Kuipers
D-J. Reijngoud

ADAPTED FROM
J BIOL CHEM. 2008 12;283(37):25437-45

ABSTRACT

Besides its well-established role in control of cellular cholesterol homeostasis, LXR has been implicated in the regulation of hepatic gluconeogenesis. We investigated the role of the major hepatic LXR isoform in hepatic glucose metabolism during the feeding-to-fasting transition *in vivo*. In addition, we explored hepatic glucose sensing by LXR upon carbohydrate refeeding.

Lxra$^{-/-}$ mice and their wild-type littermates were subjected to a fasting-refeeding protocol and hepatic carbohydrate fluxes as well as whole-body insulin sensitivity were determined *in vivo* by stable isotope procedures. *Lxra*$^{-/-}$ mice showed an impaired response to fasting in terms of hepatic glycogen depletion and TG accumulation. Hepatic G6P turnover was reduced in 9h-fasted *Lxra*$^{-/-}$ mice as compared to controls. Although hepatic gluconeogenic gene expression was increased in 9h-fasted *Lxra*$^{-/-}$ mice compared to wild-type controls, the actual gluconeogenic flux was not affected by Lxrα deficiency. Hepatic and peripheral insulin sensitivity were similar in *Lxra*$^{-/-}$ and wild-type mice. Compared to wild-type controls, the induction of hepatic lipogenic gene expression was blunted in carbohydrate-refed *Lxra*$^{-/-}$ mice, which was associated with lower plasma TG concentrations. Yet, expression of 'classic' LXR target genes *Abca1*, *Abcg5* and *Abcg8* was not affected by *Lxra* deficiency in carbohydrate-refed mice.

In summary, these studies identify LXRα as a physiologically relevant mediator of the hepatic response to fasting. However, the data do not support a role for LXR in hepatic glucose sensing.

INTRODUCTION

LXR alpha and beta (LXRα/ß; NR1H3/NR1H2) are important players in the transcriptional control of various metabolic pathways. LXRα is predominantly expressed in liver, intestine and adipose tissue, but is also present in kidney, lung, and spleen. LXRβ is expressed in almost all tissues and organs [54,55]. LXRs can be activated by oxidized cholesterol metabolites (oxysterols), which have been identified as their natural ligands. Hence, LXRs act as intracellular 'cholesterol sensors' [56]. LXRs induce lipogenic gene expression upon activation, both directly [57] and indirectly via the transcription factors SREBP-1c and ChREBP [26,57–59]. Both SREBP-1c and ChREBP control the conversion of glucose into fatty acids. Thus, LXRs coordinate the interactions between sterol and fatty acid metabolism, for instance to enable cholesterol ester formation during cellular cholesterol overload. In the past years, several studies have been published that point toward a role of LXRs in the control of glucose homeostasis. These studies showed that pharmacological LXR activation improves glycemic control in diabetic rodent models by increasing peripheral glucose disposal [60,61] and/or inhibition of hepatic gluconeogenesis [61–64]. Mitro *et al.* recently reported that physiologically relevant concentrations of either glucose or G6P are able to bind and activate LXR in HepG2 cells [53]. The physiological relevance of this potential ´glucose sensing´ role of LXR has been debated [65–67] and needs to be established.

In order to explore the physiological relevance of LXR in hepatic glucose metabolism we subjected mice deficient for *Lxra*, the major hepatic isoform, to a fasting-refeeding protocol. *Lxra*$^{-/-}$ mice showed an impaired hepatic fasting response in terms of glycogen depletion and TG accumulation. Although gluconeogenic gene expression was increased in 9-h fasted *Lxra*$^{-/-}$ mice compared to wild-type mice, stable isotope infusion revealed the actual gluconeogenic flux was not affected by Lxrα deficiency. G6P turnover was reduced in *Lxra*$^{-/-}$ mice compared to wild-type mice. In carbohydrate-refed *Lxra*$^{-/-}$ mice, the hepatic lipogenic response was blunted while changes in the expression of the LXR target genes *Abca1*, *Abcg5* and *Abcg8* were similar in wild-type and *Lxra*$^{-/-}$ mice. Taken together, these data imply an important role for LXRα in the control of hepatic glucose metabolism upon fasting but they do not support the hypothesis that LXRα acts as a hepatic glucose sensor.

EXPERIMENTAL PROCEDURES

Animals and diets

F2 male *Lxra*$^{-/-}$ mice and their wild-type littermates on a Sv129/OlaHsd C57Bl/6J mixed background [68] were housed in a light- and temperature-controlled facility (lights on 7 AM-7 PM, 21 °C). They were fed standard laboratory chow ad libitum (RMH-B, Abdiets, Woerden, The Netherlands) and had free access to water. All experiments were approved by the Ethics Committee for Animal Experiments of the University of Groningen.

Fasting and refeeding experiments

For fasting experiments we studied separate groups of mice. All mice were killed by cardiac puncture under isoflurane anaesthesia at 8 AM, either without being fasted, after a 9-h fast, or after a 24-h fast. For the refeeding experiments, mice were killed at 8 AM after a 24-h refeeding period with free access to high carbohydrate chow (38.5% w/w sucrose, Abdiets) following a 24-h fasting period.

Plasma metabolite concentrations

Blood glucose concentrations were measured using a EuroFlash glucose meter (Lifescan Benelux, Beerse, Belgium). Plasma insulin concentrations were determined using ELISA (Ultrasensitive Mouse Insulin kit; Mercodia, Uppsala, Sweden). Plasma NEFA, β-hydroxybutyrate (β-HB), TG and cholesterol concentrations were determined using commercially available kits (Roche Diagnostics, Mannheim, Germany and Wako Chemicals, Neuss, Germany).

Hepatic metabolite content and gene expression levels

Livers were quickly removed, weighed, freeze-clamped and stored at -80 °C. A small piece of liver was fixed in 4% formalin in PBS for histological analysis. Blood was centrifuged (4000 g for 10 minutes at 4 °C) and plasma was stored at -20 °C. Frozen liver was homogenized in ice-cold saline. Hepatic TG concentrations were analyzed using a commercially available kit (Roche Diagnostics) after lipid extraction according to Bligh and Dyer [69]. Hepatic G6P and glycogen content were determined as previously described [70,71]. In addition, hepatic glycogen disposition was visualized by PAS staining of 3 µm thick liver slices. RNA was extracted from frozen liver using TRI Reagent (Sigma, Zwijndrecht, The Netherlands) and subsequently converted into cDNA by a reverse transcription procedure using M-MLV and random primers according to the manufacturer's protocol. For quantitative PCR (qPCR), cDNA was amplified using the appropriate primers and probes. Primer and probe sequences for 18S, ATP binding cassette a1/g5/g8 (*Abca1/g5/g8*, *Chrebp*, *Fas*, fructose-1,6-biphosphatase 1 (*Fbp1*), *G6ph*, *G6pt*, peroxisome proliferator activated receptor gamma co-activator 1 alpha (*Pgc-1a*), *Pepck*, *Pdk4*, *Scd1* and *Srebp-1c* have been published (www.LabPediatricsRug.nl). The sequences of all other primers and probes are given in Supplemental Table 1. All mRNA levels were normalized for 18S expression.

In vivo flux measurements

Mice were equipped with a permanent catheter in the right atrium via the jugular vein [72] and were allowed a recovery period of at least three days. After the recovery period, the mice were placed in experimental cages and were fasted from 11 PM-8 AM with drinking water available. All infusion experiments were performed in conscious, unrestrained mice. To determine hepatic carbohydrate fluxes, mice were infused with a solution containing [U-^{13}C]glucose (7 µM), [2-^{13}C]glycerol (82 µM), [1-^{2}H]galactose (17 µM) and paracetamol (1 mg/mL) during six hours at an infusion rate of 0.6 mL/h as described previously [40,73]. Blood glucose concentrations were measured every 30 minutes. Blood and urine spots were collected every 60 minutes on filter paper. In total, 80-90 µL of blood was withdrawn per animal from the tail vein during these experiments.

Hyperinsulinemic euglycemic clamps were performed in a separate group of mice as described earlier [60]. Mice were fasted from 11 PM-8 AM the next day with drinking water available. During six hours, they were infused with two solutions. The first solution contained bovine serum albumin (1% w/v, Sigma), somatostatin (40 µg/mL, UCB, Breda, The Netherlands), insulin (110 mU/mL, Actrapid; Novo Nordisk, Bagsvaerd, Denmark), glucose (1111 mM) and [U-^{13}C]-glucose (33 mM, 99% ^{13}C atom %excess; Cambridge Isotope Laboratories, Andover, MA, USA) and was infused at a rate of 0.135 mL/h. The second solution consisted of glucose (1111 mM) containing [U-^{13}C]-glucose (33 mM). The infusion rate of this solution was variable to maintain euglycemia. Blood glucose concentrations were measured every 15 minutes. Every 30 minutes, a bloodspot was collected. In total, 150-170 µL of blood was withdrawn per animal from the tail vein during these experiments.

Analytical procedures for extraction of glucose from blood spots, derivatization of the extracted compounds and GC-MS measurements of derivatives were performed according to van Dijk *et al.* [39,40,73]. From this, hepatic carbohydrate fluxes were calculated using mass isotopomer distribution analysis (MIDA) as previously described [40,73]. Supplemental Figure 1 depicts the isotopic model used. To balance input and output of hepatic G6P, minor adaptations were made to the published equations [74]. The equations are given in Supplemental Table 2. Glucose production and metabolic clearance rates during hyperinsulinemic euglycemic clamps were calculated according to Grefhorst *et al.* [60].

Statistics

All data represent means ± SEM. Statistical analysis was performed using SPSS for Windows software (SPSS 12.02, Chicago, IL, USA). Analysis of data obtained in *Lxra*$^{-/-}$ versus wild-type mice was assessed by Kruskal Wallis/Mann-Whitney U-test for plasma and liver parameters. *In vivo* flux data were analyzed by ANOVA for repeated measurements. Statistical significance was reached at a p value below 0.05, except for the fasting-refeeding experiments, where this p value was adjusted for multiple comparisons.

RESULTS

We compared the changes in metabolic parameters in fasted *Lxra*$^{-/-}$ mice and wild-type littermate controls. Upon fasting, blood glucose and plasma insulin concentrations decreased while plasma NEFA and β-HB concentrations increased, without differences between *Lxra*$^{-/-}$ and wild-type mice (Table 1). Plasma TG concentrations increased upon fasting in both genotypes while plasma cholesterol concentrations were not affected. Compared to wild-type mice, hepatic G6P content tended to be higher in 9-h fasted *Lxra*$^{-/-}$ mice (Figure 1A, +73%, p=0.26). Twenty-four hours of fasting decreased hepatic G6P content in both phenotypes, but this drop was less pronounced in *Lxra*$^{-/-}$ mice. Hepatic glycogen content decreased upon fasting in both groups (Figure 1B). However, in wild-type mice hepatic glycogen content already reached its lowest level after a 9-h fast, whereas in 9-h fasted *Lxra*$^{-/-}$ mice it was similar to what observed in the fed state. Histological analysis revealed that the glycogen in the 9-h fasted *Lxra*$^{-/-}$ mice was mainly located in the periportal zone (Figure 1C). After 24 hours of fasting, hepatic glycogen stores were similarly depleted in both genotypes (Figure 1B). Hepatic TG content increased upon fasting, but to a markedly less extent in *Lxra*$^{-/-}$ mice compared to wild-type controls (Figure 1D).

Table 1. Plasma parameters in *Lxra*$^{-/-}$ mice and their wild-type littermates.

	fed		**9h-fasted**		**24h-fasted**	
	wild-type	*Lxra* $^{-/-}$	wild-type	*Lxra* $^{-/-}$	wild-type	*Lxra* $^{-/-}$
Blood glucose (mM)	8.8±0.3	9.0±0.7	5.2±0.3#	4.8±0.8#	3.5±0.4$	3.6±0.5
Plasma insulin (ng/mL)	1.59±0.38	1.37±0.58	0.12±0.03#	0.13±0.1	0.06±0.01	0.06±0.02
Plasma NEFA (mM)	0.38±0.04	0.48±0.05	0.78±0.05#	0.68±0.04	0.84±0.03	0.78±0.04
Plasma ß-HB (mM)	0.18±0.04	0.23±0.09	1.57±0.31#	1.12±0.37	3.33±0.25$	3.72±0.19$
Plasma TG (mM)	0.46±0.09	0.47±0.10	0.79±0.09	1.01±0.25	1.25±0.09$	0.91±0.17
Plasma cholesterol (mM)	1.8±0.1	1.6±0.1	2.4±0.1#	1.9±0.1*	1.8±0.2	2.4±0.4
Blood glucose (mM)	8.8±0.3	9.0±0.7	5.2±0.3#	4.8±0.8#	3.5±0.4$	3.6±0.5
Plasma insulin (ng/mL)	1.59±0.38	1.37±0.58	0.12±0.03#	0.13±0.1	0.06±0.01	0.06±0.02
Plasma NEFA (mM)	0.38±0.04	0.48±0.05	0.78±0.05#	0.68±0.04	0.84±0.03	0.78±0.04

Values represent means ± SEM for n=4-6; # p<0.05 9-h fasted *vs.* fed, $ p<0.05 24-h fasted *vs.* 9-h fasted, * p<0.05 *Lxra*$^{-/-}$ *vs.* wild-type (Mann-Whitney U-test, p value adjusted for multiple comparisons).

Gluconeogenic flux plays an essential role in glycogen accumulation [75] and hepatic gluconeogenic gene expression, *e.g.* of *Pepck* and G6pase, has been shown to be decreased upon LXR activation [61–63]. We therefore determined whether the increased hepatic glycogen content in the 9-h fasted *Lxra*$^{-/-}$ mice was paralleled by an increased expression of genes encoding enzymes involved in hepatic gluconeogenesis. Compared to wild-type mice, hepatic expression of *Pgc-1α*, *Pepck*, *Fbp1* and *G6ph* (encoding G6P hydrolase, one component of the multi-protein complex G6Pase) were all increased in 9-h fasted *Lxra*$^{-/-}$ mice (Figure 2A). Expression of genes encoding other major enzymes involved in hepatic carbohydrate metabolism (*G6pt*, *Gk*, *Pk*, *Pdk4* and *Gp*, except for *Gs* Figure 2A/B) was not affected by *Lxra* deficiency. Moreover, the lipogenic gene expression profile was similar in 9-h fasted wild-type and *Lxra*$^{-/-}$ mice, except for a reduction of *Acc2* and *Scd1* expression (Figure 2C).

Impaired hepatic G6P metabolism in 9-h fasted Lxra$^{-/-}$ mice is associated with decreased glucose turnover and increased hepatic G6P content

A 9-h fast uncovered major differences in hepatic adaptive response between wild-type and *Lxra*$^{-/-}$ mice. To determine whether the increased gluconeogenic gene expression was a cause of the observed differences in hepatic glycogen and G6P content between 9-h fasted wild-type and *Lxra*$^{-/-}$ mice, we determined glucose turnover, disposal and individual hepatic carbohydrate fluxes using stable isotope techniques [40,76]. During the infusion of the stable isotopes, blood glucose concentrations were lower in *Lxra*$^{-/-}$ mice compared to wild-type littermates (Figure 3A). Steady state isotope enrichment was reached from three hours of infusion onwards. Isotope dilution data during this steady state situation are shown in Table 2. Glucose cycling and endogenous glucose production were reduced in *Lxra*$^{-/-}$ mice compared to their wild-type littermates (Figure 3B), resulting in a decreased total glucose production. Metabolic glucose clearance was similar in both groups of mice (Figure 3C).

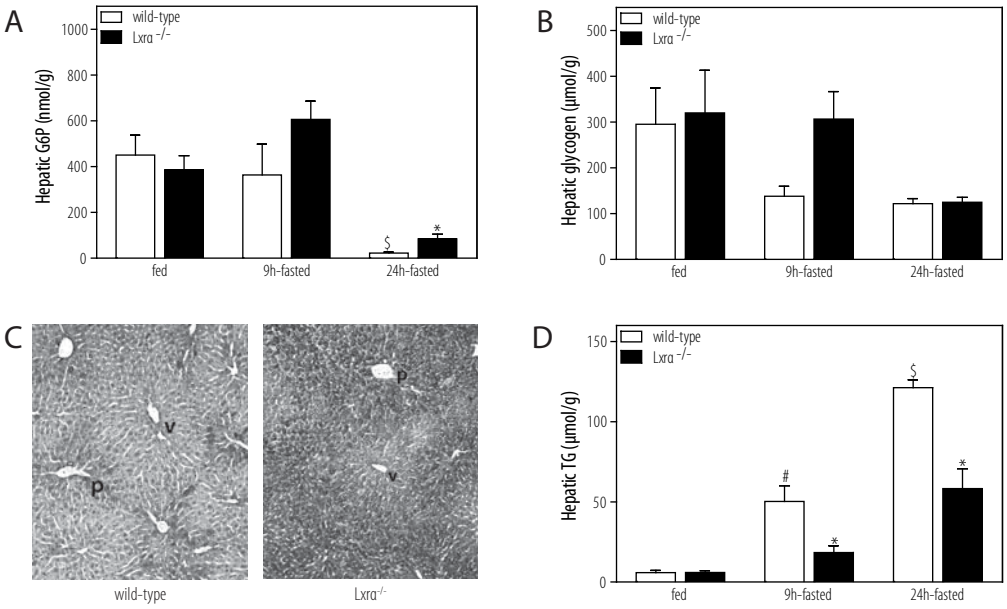

Figure 1. Fasting response in *Lxra*$^{-/-}$ mice and their wild-type littermates.
A, Hepatic G6P content. B, Hepatic glycogen content. C, Hepatic glycogen content and localization in 9-h fasted mice. P, periportal; V, perivenous and D, Hepatic TG content.
Open bars, wild-type mice; filled bars, *Lxra*$^{-/-}$ mice. Values represent means ± SEM for n=4-6; # p <0.05 9-h fasted *vs.* fed, $ p <0.05 24-h fasted *vs.* 9-h fasted, * p <0.05 *Lxra*$^{-/-}$ *vs.* wild-type (Mann-Whitney U-test, p value adjusted for multiple comparisons).

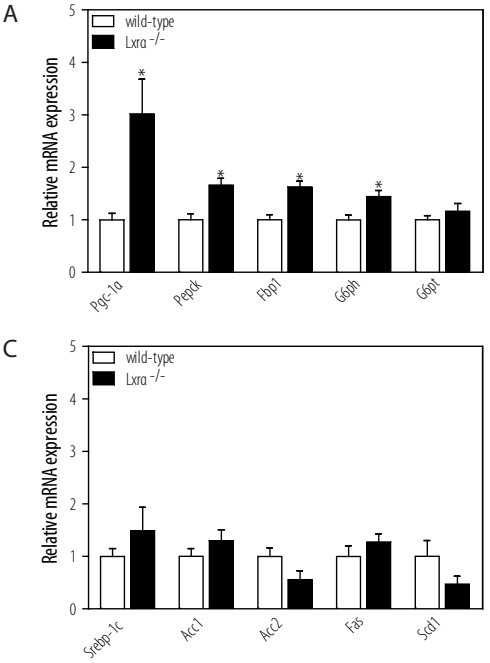

Figure 2. Hepatic gene expression levels in 9-h fasted *Lxra*$^{-/-}$ mice and their wild-type littermates.
A, Gluconeogenic gene expression. B, Glycolytic gene expression and C, Lipogenic gene expression.
Open bars, wild-type mice; filled bars, *Lxra*$^{-/-}$ mice. Values represent means ± SEM for n=5; * $p<0.05$ *Lxra*$^{-/-}$ *vs.* wild-type (Mann-Whitney U-test).

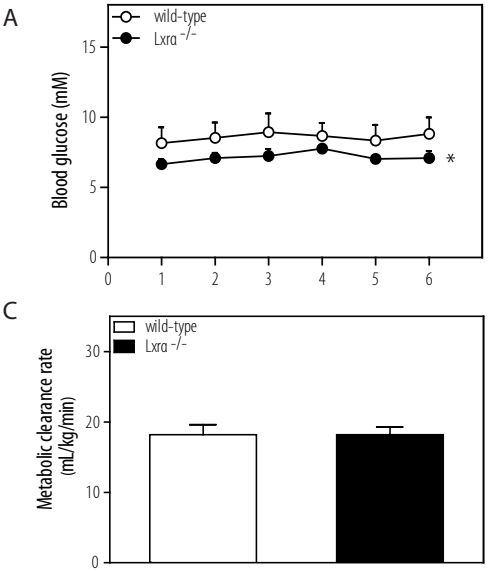

Figure 3. Whole-body glucose fluxes in 9-h fasted *Lxra*$^{-/-}$ mice and their wild-type littermates during steady state infusion (t=180-360 min).
A, Blood glucose concentrations during isotope infusion. Open dots, wild-type mice; filled dots, *Lxra*$^{-/-}$ mice. B, Total glucose production and contribution of endogenous glucose production (dark grey bars) and glucose cycling (light grey bars) and C, Metabolic glucose clearance rates. Open bars, wild-type mice; filled bars, *Lxra*$^{-/-}$ mice. Values represent means ± SEM for n=6; * $p<0.05$ *Lxra*$^{-/-}$ *vs.* wild-type (ANOVA).

Table 2. Primary isotopic parameters during steady state infusion (t=180-360 min) in 9-h fasted *Lxra*$^{-/-}$ mice and their wild-type littermates.

	wild-type	*Lxra* $^{-/-}$
Isotope dilution		
d(glc)	0.016±0.001	0.019±0.001*
d(UDPglc)	0.141±0.008	0.164±0.006*
Isotope exchange		
c(glc)	0.27±0.02	0.19±0.01*
c(UDPglc)	0.15±0.03	0.11±0.01
MIDA analysis		
f(glc)	0.66±0.03	0.71±0.02
f(UDPglc)	0.54±0.02	0.52±0.01

Values represent means ± SEM for n=6; * $p<0.05$ *Lxra*$^{-/-}$ *vs.* wild-type (ANOVA).
For abbreviations see Supplemental Table 2.

Gluconeogenic flux, *i.e.*, *de novo* synthesis of G6P was not affected by Lxrα deficiency (Table 3). In addition, the compartmentation of newly synthesized G6P towards glucose (86±1% in both *Lxra*$^{-/-}$ and wild-type mice) and glycogen (14±1% in both *Lxra*$^{-/-}$ and wild-type mice) was comparable in both genotypes. However, glucose phosphorylation (glucokinase flux), dephosphorylation (glucose-6-phosphatase flux), glycogen synthesis (glycogen synthase flux) and glycogen breakdown (glycogen phosphorylase flux) were reduced in *Lxra*$^{-/-}$ mice compared to wild-type mice (Table 3). G6P turnover and glucose balance were reduced in *Lxra*$^{-/-}$ mice compared to wild-type littermates, while glycogen balance tended to be less negative in *Lxra*$^{-/-}$ mice (Figure 4).

Table 3. Individual fluxes comprising hepatic G6P metabolism during steady state infusion (t=180-360 min) in 9-h fasted *Lxra*$^{-/-}$ mice and their wild-type littermates.

	wild-type	*Lxra* $^{-/-}$
Gluconeogenic flux	109±6	98±4
Glucokinase flux	75±9	39±3*
Glucose-6-phosphatase flux	223±16	158±6*
Glycogen synthase flux	45±4	34±2*
Glycogen phosphorylase flux	71±7	48±4*
c(UDPglc)	0.15±0.03	0.11±0.01

Values represent means in µmol/kg/min ± SEM for n=6; * $p<0.05$ *Lxra*$^{-/-}$ *vs.* wild-type (ANOVA).

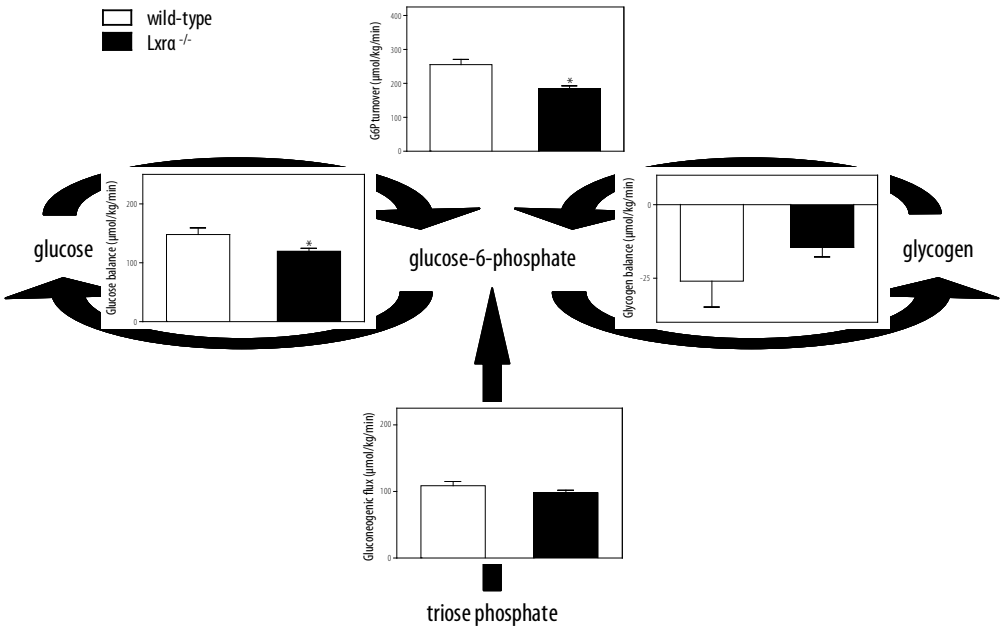

Figure 4. Hepatic glucose balance, glycogen balance, G6P turnover and gluconeogenic flux in 9-h fasted *Lxra*$^{-/-}$ mice and their wild-type littermates during steady state infusion (t=180-360 min).
Open bars, wild-type mice; filled bars, *Lxra*$^{-/-}$ mice. Values represent means ± SEM for n=6; * $p<0.05$ *Lxra*$^{-/-}$ *vs.* wild-type (ANOVA).

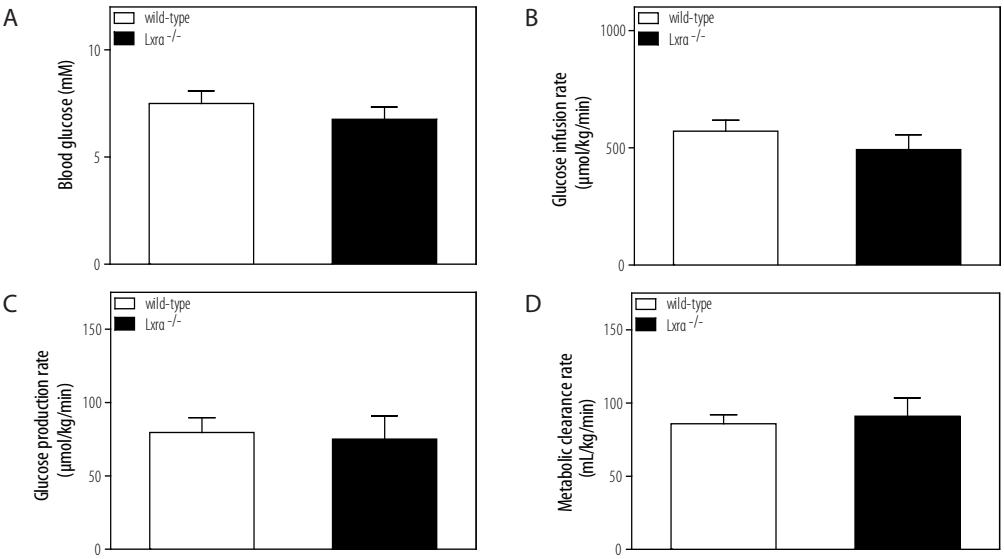

Figure 5. Glucose metabolism under hyperinsulinemic euglycemic clamp conditions in 9-h fasted *Lxra*$^{-/-}$ mice and their wild-type littermates during steady state infusion (t=180-360 min).
A, Blood glucose concentrations. B, Glucose infusion rates required to maintain euglycemia. C, Endogenous glucose production rates and D, Metabolic glucose clearance rates.
Open bars, wild-type mice; filled bars, *Lxra*$^{-/-}$ mice. Values represent means ± SEM for n=5.

Hepatic and peripheral insulin sensitivity are maintained in Lxra$^{-/-}$ mice

Insulin is a major regulator of carbohydrate metabolism. Although plasma insulin concentrations did not differ between 9-h fasted wild-type and *Lxra*$^{-/-}$ mice (Table 1), we questioned whether insulin sensitivity of hepatic and peripheral glucose metabolism was altered in *Lxra*$^{-/-}$ mice. We therefore performed hyperinsulinemic euglycemic clamps in 9-h fasted conscious, unrestrained mice. Steady state isotope enrichment and euglycemia (Figure 5A) were reached within three hours of infusion. The glucose infusion rates to maintain euglycemic conditions (Figure 5B) did not differ between the two genotypes, indicative for unaffected whole-body insulin-sensitivity in *Lxra*$^{-/-}$ mice compared to wild-type littermates. Hepatic insulin sensitivity was not affected in *Lxra*$^{-/-}$ mice. Hyperinsulinemia resulted in a 41% and 51% reduction of hepatic glucose production in *Lxra*$^{-/-}$ and wild-type mice, respectively (compare Figure 5C with Figure 3B). In addition, peripheral insulin sensitivity was not affected by Lxrα deficiency since the MCR was increased to 406% in *Lxra*$^{-/-}$ mice and 378% in wild-type littermates (compare Figure 5D with Figure 3C).

Carbohydrate refeeding affects hepatic lipogenesis and gene transcription independent of LXRα

We also determined whether there are indications for glucose-mediated LXR activation. Therefore, plasma and liver metabolite concentrations were assessed in *Lxra*$^{-/-}$ and wild-type mice that were refed a carbohydrate rich diet following a 24-h fast (Table 4). Blood glucose and plasma insulin, NEFA and β-HB concentrations were comparable in both groups of carbohydrate-refed mice. Plasma TG concentrations were lower in carbohydrate-refed *Lxra*$^{-/-}$ mice compared to wild-type mice, while plasma cholesterol concentrations were similar. Hepatic G6P and glycogen content were increased in carbohydrate-refed mice compared to mice that had been fasted for 24 hours (Figures 2A and 2B) but no differences were observed between the two genotypes (Table 4). Hepatic TG content was lower in carbohydrate-refed *Lxra*$^{-/-}$ mice (p=0.052).

Table 4. Plasma and liver parameters upon refeeding in *Lxra*$^{-/-}$ mice and their wild-type littermates.

	wild-type	*Lxra*$^{-/-}$
Blood glucose (mM)	9.5±0.5	9.8±0.6
Plasma insulin (ng/mL)	1.66±0.49	2.74±0.66
Plasma NEFA (mM)	0.34±0.01	0.30±0.02
Plasma ß-HB (mM)	0.11±0.01	0.12±0.01
Plasma TG (mM)	3.00±0.18	1.78±0.23*
Plasma cholesterol (mM)	3.7±0.2	3.3±0.1
Hepatic G6P (nmol/g)	347±23	359±41
Hepatic glycogen (µmol/g)	1121±81	1088±91
Hepatic TG (µmol/g)	18.1±1.5	13.0±1.6

Values represent means ± SEM for n=5-6, * $p<0.05$ *Lxra*$^{-/-}$ *vs.* wild-type (Mann-Whitney U-test).

In both groups of mice, carbohydrate refeeding increased expression of *Gk*, *Pk*, and *Gp*, while *Pdk4* expression was decreased. *Chrebp* and *Gs* expression were not affected by carbohydrate refeeding (Figure 6A). Expression of *Srebp-1*c, *Acc1*, *Fas* and *Scd1* was clearly induced in carbohydrate-refed wild-type mice, but this response was less pronounced in *Lxra*$^{-/-}$ mice. *Acc2* expression was not affected by carbohydrate refeeding (Figure 6B). In both wild-type and *Lxra*$^{-/-}$ mice, expression of the LXR target genes *Abca1*, *Abcg5* and *Abcg8* was not induced by carbohydrate-refeeding (Figure 6C).

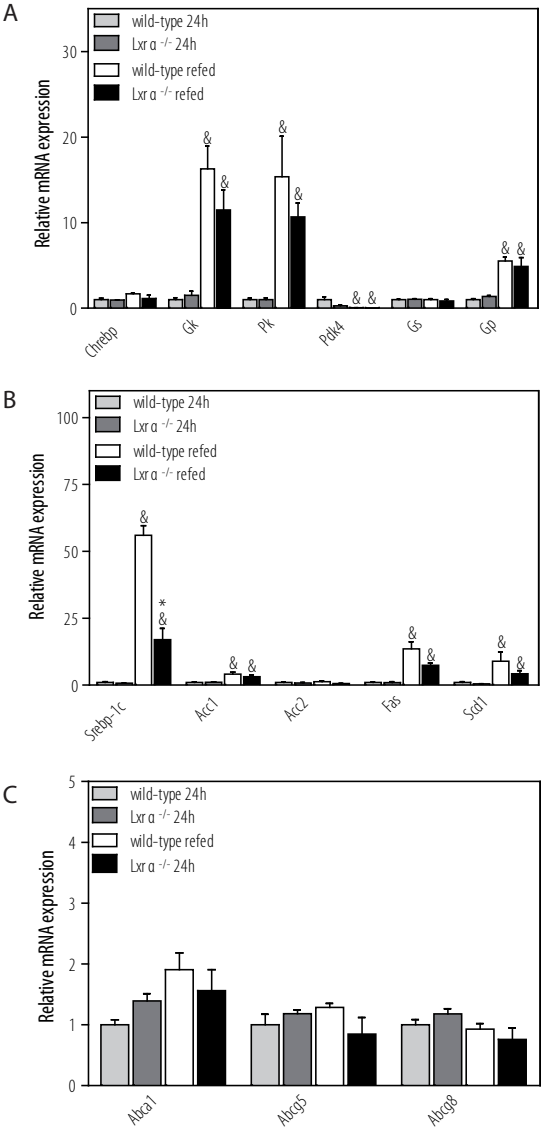

Figure 6. Hepatic gene expression levels upon fasting and refeeding in *Lxra*$^{-/-}$ mice and their wild-type littermates.
A, Glycolytic gene expression. B, Lipogenic gene expression. C, Cholesterol transporter gene expression.
Light grey bars, 24-h fasted wild-type mice; dark grey bars, 24-h fasted *Lxra*$^{-/-}$ mice; open bars, refed wild-type mice; filled bars, refed *Lxra*$^{-/-}$ mice. Values represent means ± SEM for n=5; & $p<0.05$ refed *vs.* 24-h fasted * $p<0.05$ *Lxra*$^{-/-}$ *vs.* wild-type (Mann-Whitney U-test, p value adjusted for multiple comparisons).

DISCUSSION

LXRs act as cholesterol sensors that control transcription of genes involved in cellular cholesterol and lipid homeostasis. Lipid and carbohydrate metabolism are tightly linked and strongly regulated to ensure an adequate control of whole-body energy metabolism. LXR regulates transcription and activity of the glucose-sensing lipogenic transcription-factor ChREBP [57], which strongly suggest a physiological role of LXR in hepatic carbohydrate metabolism in the postprandial state. It is known that LXR activation results in hepatic steatosis [51,58]. On the other hand, prolonged fasting is also associated with hepatic lipid accumulation [9]. These lines of evidence prompted us to study the role of hepatic LXR during fasting and refeeding. LXRα is considered to be the major isoform regulating lipogenic gene expression in the liver. Therefore, we subjected *Lxra*$^{-/-}$ mice [68] to fasting and refeeding protocols and we applied sophisticated stable isotope techniques to quantify hepatic carbohydrate fluxes *in vivo* in these mice.

We are the first to show that *Lxra* plays an important role in the feeding-to-fasting transition. *Lxra* deficiency results in an impaired fasting response, indicated by a delayed fasting-induced hepatic glycogen depletion and increased hepatic G6P content in 9-h fasted *Lxra*$^{-/-}$ mice compared to wild-type littermates. Moreover, the *Lxra*$^{-/-}$ mice accumulated less hepatic TG upon fasting. Expression of gluconeogenic genes was increased in 9-h fasted *Lxra*$^{-/-}$ mice compared to wild-type littermates. This is in agreement with the decreased expression of *Pgc-1α*, G6pase and *Pepck* upon pharmacological LXR activation [61–63]. However, evaluation of hepatic carbohydrate fluxes in 9-h fasted mice revealed that the induction of gluconeogenic gene expression in *Lxra*$^{-/-}$ mice was not paralleled by an increased gluconeogenic flux. Thus, there is a discrepancy between gene expression levels and gluconeogenic flux *in vivo* [60]. This indicates that other factors such as precursor availability [77,78] and post-transcriptional modification of enzymes are important determinants that control hepatic carbohydrate fluxes *in vivo*.

Glucose phosphorylation and dephosphorylation as well as glycogen synthesis and breakdown were reduced in *Lxra*$^{-/-}$ mice compared to wild-type littermates. Thus, instead of an altered *de novo* synthesis of G6P the inter-conversions of G6P, glucose and glycogen were clearly affected in 9-h fasted *Lxra*$^{-/-}$ mice. The net effect of the lower glycogen synthesis (-24%) and breakdown (-32%) fluxes in *Lxra*$^{-/-}$ mice was a less negative glycogen balance, supporting the delayed glycogen depletion observed upon fasting in the *Lxra*-deficient mice. The remaining glycogen was located in the periportal zone. It is known that upon fasting, glycogen is initially degraded to G6P in periportal hepatocytes. In perivenous hepatocytes, glycogen is predominantly broken down into pyruvate and hence released as lactate (reviewed in [79]). Thus in the livers of 9-h fasted *Lxra*$^{-/-}$ mice, less glycogen was broken down, contributing to the reduced G6P turnover observed in these mice. The changes in G6P and glycogen metabolism were not secondary to changes in hepatic gluconeogenesis [75,80], since neither the gluconeogenic flux nor the partitioning of newly synthesized G6P towards glucose and glycogen was affected by *Lxra* deficiency. In addition, the net effect of the lower glucokinase and glucose-6-phosphatase fluxes was a reduction in endogenous glucose production and glucose cycling.

Glycogen synthesis and breakdown are regulated by several factors including insulin. Although insulin concentrations were comparable in 9-h fasted *Lxra*$^{-/-}$ mice and their wild-type littermates, hepatic insulin sensitivity could have been altered by Lxrα deficiency, explaining the differences

observed in hepatic G6P and glycogen content as well as their inter-conversions. Hepatic and peripheral insulin sensitivity were determined in 9-h fasted *Lxra*$^{-/-}$ mice and their wild-type littermates using hyperinsulinemic euglycemic clamps. Insulin sensitivity of both hepatic glucose production and peripheral glucose disposal was not affected by Lxrα deficiency. Although LXR agonists have been implicated as potential insulin sensitizers [61,62,64], our data do not support a direct role of LXR as a potential mediator of hepatic and peripheral insulin sensitivity [60]. However, many of the studies performed on the role of LXR are based on pharmacological activation. In the *Lxra*$^{-/-}$ mice there may be some adaptations that prevent the endogenous ligand from increasing, of there may be additional systems that compensate for the Lxrα deficiency. The reduced hepatic carbohydrate fluxes could also be a result from an altered reliance on glucose versus fatty acids and/or a differential energy demand in the *Lxra*$^{-/-}$ mice during the feeding-to-fasting transition. In addition to the delay in glycogen depletion observed upon fasting in the *Lxra*$^{-/-}$ mice, these mice accumulated remarkably less TG. Gene expression analysis provided indications for an increase in hepatic fatty acid oxidation in fasted *Lxra*$^{-/-}$ mice, which could explain this remarkable reduction in hepatic TG accumulation (data not shown). However, additional *in vivo* studies are required to determine the physiological relevance of these observations.

Finally, we explored the role of LXRα in glucose-induced hepatic lipogenesis. Upon refeeding, hepatic TG content was lower and plasma TG levels were reduced in *Lxra*$^{-/-}$ mice compared to wild-types. Quite strikingly, no differences in *Chrebp* expression were observed between *Lxra*$^{-/-}$ and wild-type mice. This is in contrast to observations by Cha and Repa (4) which suggested that CHREBP is a downstream target of LXRα. However, carbohydrate refeeding resulted in a less pronounced induction of *Srebp-1*c and other lipogenic gene expression in the *Lxra*$^{-/-}$ mice compared to the wild-types. Considering our observation that *Chrebp* and *Pk* expression were similar in carbohydrate-refed *Lxra*$^{-/-}$and wild-type mice, we conclude that the blunted lipogenic response in carbohydrate-refed *Lxra*$^{-/-}$ mice resulted from the reduced SREBP-1c activity secondary to Lxrα deficiency. Apparently, the relationship between LXR, ChREBP and SREBP-1c on the one hand and hepatic TG metabolism on the other hand requires further investigation.

Recent in vitro studies have shown that glucose is able to bind and activate hepatic LXR [53], suggesting that LXR may act as a putative hepatic 'glucose sensor'. However, the physiological relevance of glucose sensing by LXR has been debated [65–67] and therefore required further investigation. In the studies performed by Mitro *et al.* [53], the expression of the cholesterol transporters that are direct LXR targets, *e.g.*, *Abca1* and Abcg1 only marginally increased upon carbohydrate-refeeding, whereas lipogenic mRNA expression was clearly induced.

We confirmed that the expression of the 'classic' LXR-target genes *Abca1*, *Abcg5*, and *Abcg8* was not affected by carbohydrate-refeeding in *Lxra*$^{-/-}$ mice. Thus, the effect of carbohydrate refeeding on hepatic lipogenic gene expression was different from that on expression of the cholesterol transporters *Abca1*, *Abcg5*, and *Abcg8*. Similar results have been obtained by Denechaud *et al.* [67], who showed no induction of hepatic *Abcg1* and *Abca1* mRNA expression in carbohydrate-refed mice while lipogenic gene expression was induced. Moreover, in contrast to the blunted induction of lipogenic gene expression, Abcg1 and *Abca1* expression was not affected in carbohydrate-refed *Lxraβ*$^{-/-}$ mice compared to wild-type controls [67]. Taken together, these and our data provide strong evidence that carbohydrate refeeding does not induce hepatic gene expression via LXR, and there-

fore question the physiological relevance of glucose sensing by hepatic LXR *in vivo*.

In summary, our data identify LXRα as an important player in control of metabolic adaptation during the feeding-to-fasting transition, but question the physiological relevance of glucose sensing by hepatic LXR. In addition to its regulatory role in cholesterol, lipid and glucose metabolism to ensure energy storage in the postprandial state, LXRα seems to facilitate the release of stored energy upon fasting. Under these conditions, LXRα not only mediates TG accumulation, but also controls hepatic G6P and glycogen deposition as it determines the partitioning and turnover of these energy-bearing molecules, possibly to fulfill the liver's demand of these metabolites.

ACKNOWLEDGEMENTS

The authors thank Juul F.W. Baller for excellent technical assistance.